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Discussion: Session 4* New Approaches to Detecting Low-Dose Effects

Dr. Radford began the discussion by asking Dr. Christian if he could give a quick survey of the types of environmental exposures that he believes are most amenable to twin studies and give examples.

Dr. Christian replied that he thought the major use of this method is to control familial sources of variation and try to get more efficient studies. Evaluation of traits which are under more genetic control, for example, would be very useful. A recent survey of twin studies of 11 different drugs revealed that all had highly significant components of genetic variance. This would be a fruitful area to study. Another criterion is relatively short-term effects, for example, his laboratory is trying to determine the effects of salt on blood pressure. In general, one can survey traits of interest just by uniformity trials, to get an idea of how correlated monozygotic twins are. He added that there have not been a lot of studies of general environmental agents by the twin method.

Dr. Radford commented that the twin registry of the Veterans Administration has been around a long time. One of the concerns of the people who maintain the registry has been that the registry will die out before anybody really uses it properly. It may be as Dr. Christian pointed out, that ascertaining the twin status in a study will be a very useful adjunct to the study. On the other hand, if one is really going to use the twin method, only by resorting to twin registries will the numbers be big enough. Otherwise, the twin sample may not be sufficient to pick up the kinds of subtle effects that low-level exposures to environmental agents may produce. He asked Dr. Christian whether there ought to be a recommendation that money be budgeted to set up a twin registry on a continuing basis, as has been done, for example, in Sweden. Dr. Christian commented that people may not be aware of the World War II Veterans twin registry. It is sponsored and kept track of by the National Academy of Sciences and the National Research Council. They have an Advisory Committee on which he is serving at the present time. There has indeed been concern that the registry has been underutilized. These twins are now 55 years of age on the average, with a range of about 10 years of age, and the registry consists of about 15,000 pairs of twins. He has reviewed the status of all twin registries in the United States and Canada and he thought there are about 31 panels available. Most of these are ascertained because they are twins. To use these to look for specific risk factors or specific diseases may result in fairly low yield, even though some of the panels are quite large. The largest twin panels in the world are in the Scandinavian countries. This is primarily because of their national health insurance which allows them to keep track of people.

He had tried to stress, however, that another resource had not been tapped, and that is the case where twins are ascertained, not because they are twins, but because they are from a relatively large group of people who are at risk. He thought this opportunity has really not be exploited. He and his colleagues are doing a study on hypertension and are trying to develop a twin panel of this type. He believed the yield of twins at risk is likely to be higher if one is studying several thousand individuals who are at high risk from some sort of environmental cause or disorder, than for twin registries that are maintained because they are twins. He also thought that bias of ascertainment might be lower for twin subsets of a general

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population than for twin registries. He emphasized that it is very easy to ascertain whether a person is a twin or not, and therefore he urged those in the audience to keep track of twins in their studies.

Mr. David Goldsmith (Research Triangle Institute, North Carolina) asked Dr. Conner if there was a possibility that some of the false positives from sister chromatid exchange could result from such heavy exposures that no cells would be dividing. Secondly, could she given an estimate of the proportion of false negatives that might be found for sister chromatid exchange. He thought this measure of validity was an important contribution of the complete Ames test protocol. However, for other kinds of short-term assays there has not been a well-organized attempt to determine the sensitivity and specificity of the tests.

Dr. Conner replied that with regard to false positives, Mr. Goldsmith was correct in that with a relatively high acute dose, if the agent is in fact lethal to the cells, that would distort the population that would be scored, since any cells that are lost cannot be scored. But she thought that this source of error could be detected, for example, by doing a kinetic analysis at various times after exposure. Laboratory tests of dose-response to the particular chemical are the best way to deal with this problem.

With regard to negative results, one agent which she had mentioned was radiation, which is not very effective as a sister chromatid exchange inducer. Agents such as bleomycin cause the same basic damage as x-rays and are also not very effective in the SCE assay. Concerning the relative sensitivity and specificity of the Ames test and other tests, she considered that no matter what mutagen assay test system was used, each has its own specific sensitivity and specificity. She thought these should be determined in terms of the reaction of chemical groups, allowing one to group chemicals according to structure-reactivity.

Dr. Philip Archer (University of Colorado Medical School) commented that he had been participating in the screening of workers for chromosomal abnormalities for some time, and while they found a radiation dose-response, he agreed with Dr. Conner about the cost justification for this approach. It is very costly and probably is not justified for other than research purposes or possibly in cases of very heavy exposures. Their group has cut back from 100 cells per person to about 30 cells and they have stockpiled the unread slides, essentially establishing a potential data base for possible future reference. He asked Dr. Conner to comment on her allusion to an effect of age on frequency of chromosomal abnormalities. They have not found such an effect in their control groups. He also asked whether a new banding technique, in which the cells are stopped at a different part of the cycle, could be used as a screening tool for detecting effects of environmental exposures.

Dr. Conner addressed the second question. She thought the new technique is used primarily by Yunis. She was not familiar with any application in population screening, but she believed it may appear to be more promising than it really might be, because she thought it would be difficult to obtain the proper cell population sizes with clear chromosome elongation and banding patterns, although the fact that one obtains more banding patterns may give you more information concerning a specific type of damage.

Dr. Neil Wald (University of Pittsburgh) responded to the question about the effect of age on chromosome aberrations. In Evans' study of nuclear shipyard workers exposed to low radiation doses, he demonstrated an effect of age, but he did not indicate whether he considers this effect could be due to accumulation of background radiation exposure which also increases with age. Another factor could be medical diagnostic x-ray exposure that also increases with age.

Dr. S. Raman (University of Ottawa) asked Dr. Conner about the problem of comparative cytogenetic results from different laboratories and the possible reasons for differences found in controls. He also commented that since some cytogenetic responses are transient they can be modified by a number of factors, such as smoking and drugs. In this case, there may be little specificity of effects observed.

Dr. Conner replied that with regard to variation among laboratories, some of the factors that she had mentioned, such as smoking and other factors, were not taken into consideration by all the laboratories. But more importantly, she thought the technique was still new enough that all of the factors that contribute to increased exchange levels have not been well defined. A recent paper has suggested that the concentration of the BrDU analog in the culture medium is more important in inducing baseline SCE's than that incorporated into the chromosomes. Some of the reasons for this effect are going to have to be investigated further before we can reduce the variability between labs. The makeup of the cell culture medium may be very critical. With regard to the specificity of their techniques, she should have mentioned that in the Livermore study, in particular, all of the individuals in the study population were extensively interviewed to determine smoking habits, whether they

had been on any kind of medication, had viral diseases, and so forth. Those individuals who gave a positive history were removed from both the control and exposed populations before the final comparison was done. She thought this was very important, and these issues were going to have to be addressed in order to remove possible confounding factors.

Dr. Gordon Newell (Assembly of Life Sciences, National Research Council) commenting on the reliability of data, stated that the Environmental Mutagen Society is finally addressing this problem. He strongly urged that those employing these short-term tests make sure the laboratory can produce reliable and consistent results. He cited a major study of 22 pairs of compounds that are being evaluated blind by about 15 laboratories using the Ames test. He thought that about 15% of the compounds tested had results widely different among the test laboratories. It is these kinds of difficulties that give great concern to people when new procedures come along. He hoped that someone will take the responsibility in a central organization and distribute some unknowns on a regular basis, as is done for clinical laboratories. A procedure that might be worthwhile is called the host-mediated assay in which, after exposure to a known mutagen, urine is tested for breakdown products and conjugates, and these are assayed for mutagenicity. This technique has a fair amount of reliability.

Dr. Joseph H. Meyer (E.R. Squibb & Sons, Princeton, New Jersey) asked Dr. Petrakis to speculate on the possible role of genetic interaction with some drug effects that are postulated. For example, the question of reserpine causing breast cancer; vaginal cancer in daughters of mothers who had estrogens during pregnancy; estrogen and endometrial cancer or birth defects. Scientific evidence on these issues comes from retrospective studies which figure very prominently as legal evidence. These retrospective studies have not paid heed to possible genetic factors. He wondered what role genetics, in hindsight, could play in these situations. Dr. Petrakis replied that he had mentioned pharmacogenetic studies. For example, twin studies using pharmacogenetic approaches have shown some things one could learn about genetic-environmental interaction. At present these matters are still largely speculative. The method that Dr. Tokuhata showed and that he had also used, may be a way to look at this question of genetic-environmental interaction. He thought one could look at any condition where there is familiality and try to examine some of the factors of interest. The problem here will be the need for sufficient numbers in each of the groups to enable one to do a good epidemiological analysis. Dr. Christian commented that among all of the drugs that he had looked at, there was a spectrum of genetic variability among people studied, not only affecting toxicity but also effectiveness. Some people genetically are such that they do not respond as one might expect them to. Many studies of this type of relationship are retrospective and prospective studies will be helpful. When Dr. Schneiderman was talking yesterday about needing 20 years of exposure and 20 years of follow-up for his work, we may need to follow these patients and their descendents for five generations!

Dr. Turner stated that for epidemiologists the attractiveness of monozygotic (MZ) twins in a study is very great. However, there are aspects of the biology of twinning which suggest that caution should be applied to generalizing from studies of twins because of developmental differences between MZ twins which can be substantial. Thus studies which define increased risks between monozygotic twins, in an epidemiologic sense, may be primarily relatable to embryogenesis of the twins. Many geneticists tend to think that MZ twins are superior instruments for study when in fact they are not. The question is: to what degree does the lateralization of the developmental process of the MZ twin interfere with or weaken inferences, as one would draw them, when one compares twins with paired members from the nontwin population.

Dr. Christian agreed that this is a major problem. In classic twin studies, when we compare the two types of twins and the trait itself may be associated with one twin or the other, for those associated with the MZ twins there may be lateralization effects. One striking example is that there are more left-handed twins than there are left-handed singletons, and left-handed twins are most likely the psychologically dominant twin. A recent study from his laboratory, published with all sorts of disclaimers, showed that in pairs of identical twins in which there was a left-handed and a right-handed twin, the left-handed one was born first 80% of the time. A Finnish twin panel found the same result. What it means in terms of twin studies is unclear. For the type of studies he had discussed, lateralization is not particularly important because there is a built-in measure of the concordance or the similarity of these twins. That is why he emphasized that to study any trait one should first see how similar these twins are, and if one finds MZ twins are markedly different for one reason or another, then the study might teach something about twins, but it is not likely to tell much about the drug.